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DEVELOPMENT OF QUALITY STANDARDS OF *HOLARREHENA ANTIDYSENTERICA* (LINN.) BARK

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Abstract

Holarrehena antidysenterica (Linn.) Wall belonging to family Apocynaceae is a deciduous laticiferous shrub or small tree, growing wild throughout the Himalayan region of India. It is commonly used in day to day life. In present investigation an attempt has been made for the pharmacognostical standardization and evaluation of *H. antidysenterica* bark. The pharmacognostical evaluation comprises of detailed macroscopy, powdered microscopy, fluorescence analysis and physical constants such as ash and extractive values. The bark extracts were subjected to preliminary phytochemical screening. The data obtained in present study will serve as valuable tool for identification, authentication and detection of adulterants, standardization and quality control of the drug. The developed technique will also be useful for the standardization of formulations containing *H. antidysenterica*

Keywords: *H. antidysenterica*, Extractive values, Ash values

Introduction

Holarrehena antidysenterica (Linn.) Wall belonging to family Apocynaceae commonly known as kutaja or kurchi(Malyalam, India) distributed in Asia, tropical areas of Africa, Madagascar, India, Philippines and Malayan Peninsula. Growing up to an altitude of 13,00 m in the Himalayas, often gregariously in deciduous forests, open waste lands and abundant in sub-Himalayan tract [1]. The plant has been employed for long time in folklore therapy. 'Kurchi' bark is an important traditional drug used in various ailments. The drug is astringent, anthelmentic, stomachic, antipyretic, tonic and is generally administered as an extract or decoction in amoebic dysentery and diarrhoea. Bark is given either alone or with other astringent drugs in piles, colic, dyspepsia, chest affections and diuretics; also reported to be useful in skin diseases and spleen. A hot decoction of the drug is used as a gargle in toothache [2,3,4,5]. It consists certain biochemical constituents namely alkaloids, glycosides ,phenolic compounds and tannins. The bark has been reported to have antitubercular, amoebicidal, antiprotozoal, anticancerous and hypoglycaemic [6]. The drug is collected from the wild sources and varies in constituents and efficacy due to the geographical diversity. Improper collection and storage condition lead to the deterioration of the raw material. Keeping in view the above mentioned problems, it was essentials to standardize the bark of *H. antidysenterica* for the establishment of quality and identity profile of the drug for the purpose of safety monitoring and overall quality assurance of the drug. Since there is no report in literature regarding the standardization of *H. antidysenterica bark*. Therefore, in the present investigation an attempt has been made to standardize *H. antidysenterica* bark by using macroscopic and microscopic characters, powder microscopy, fluorescence analysis and physico-chemical values.

H. antidysenterica is a deciduous laticiferous shrub or small tree, up to 13 m high and 1.1 m in girth with clear bole of 3-7 m. Leaves 15-30 x 4-12 cm, base obtuse, rounded or acute, nerves 10-14 pairs, opposite, sessile, elliptic or ovate, oblong, membranous, strong, arched; petiole up to 1.5 cm; cymes 3-6 cm diameter. Corymbose sessile terminal; bracts small, ciliate; pedicels slender. Flowers inodorous white, in terminal corymbose cyme. Calyx-lobe 2.5-3 mm long, oblong-lanceolate, acute, ciliate. Corolla puberulous outside; tube 8-13 mm long, slightly inflated near the base over the stamens,

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mouth not closed with ring of hair; throat hair inside; lobes about equaling the tube, oblong, rounded at the apex, more or less pubescent. Follicle divaricated, cylindrical, 15-45 cm long and 5-10 mm in diameter, parallel, terete, corecious, obscurely lonelose, usually with dotted white spots. Seeds 8 mm long or more, linear oblong, tipped with spreading deciduous coma of brown hair, 2-2.5 cm long, light brown, 8-12 mm long, 900-1000 seeds weighing one ounce (Oz.), 25-30 in a follicle: coma brownish, spreading, 2.5-10 cm long [1,7].

Considerable work has been carried out on the chemistry and biological activity of 'Kurchi' bark. Alkaloids have been reported in the bark, leaf and seeds of the plant. Bark is reported to be richest resource of alkaloids, which are located in the phloem tissue and not in the periderm [8]. The alkaloid content of the bark rises with the age and girth of plant. The Indian Pharmacopoeia mentions 2% minimum limit for the total alkaloids in 'Kurchi' bark and 1% w/v in liquid extract of 'Kurchi'. The highest (4.27%) alkaloid content has been reported in the stem bark collected from Gujarat state. The adulterants of 'Kurchi' bark, *W. tomentosa* contains 1.55% [9] and 0.4% of alkaloids while that of *W. tinctoria* contain 0.23% of alkaloids [10].

Material and Methods

Chemicals and reagents

All the chemicals and reagents used were of analytical grade, purchased from Sigma chemical co. (St Louis, MQ, USA) and Merck (Darmstadt, Germany). *H. antidysenterica* bark was collected fresh from Himachal Pradesh and from the local market in delhi which was identified by Taxonomist (Professor M.P. Sharma), Department of Botany, Hamdard University New Delhi. The voucher specimen was deposited in Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard.

Morphological studies

The morphological studies were carried out for shape, size, colour, odour, taste and fracture of the *H. antidysenterica* bark.

Microscopic studies and powder analysis

The transverse section of bark was prepared by standard method. Slides of powdered bark material were also prepared and studied. Microphotography on different magnifications was carried out with motic microscopic unit. Polarized light was used for the study of crystals, starch granules and lignified cells.

Physicochemical Standardization

The various physico-chemical values of bark such as ash values, extractive values, loss on drying, were determined according to the Pharmacopoeial method.

Phytochemical screening

The phytochemical evaluation of drug was carried out as per the method described. Previously dried powdered leaves (5 gm) were extracted in a Soxlet apparatus with petroleum ether, chloroform, methanol and water successively. The extracts were evaporated to dryness under vacuum. These extract were used for the analysis of different phyto-constituents *viz*. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc.

Fluorescence Analysis

The fluorescence nature of powder drug was analyzed and the observations with different chemicals were also carried out and recorded.

Results and Discussion

Macroscopical evaluation

The samples of 'kurchi' bark were collected fresh from Himachal Pradesh and from the local market. Market samples were in pieces of 2-8 cm long, 1.5-5 cm broad and up to 1-9 mm thick; these were recurved both longitudinally and transversely. The outer surface is grayish-brown to reddish-brown, shows longitudinal cracks and bears horizontal lenticels. The inner surface is rough, longitudinally striated, frequently with portion of pale-yellow wood attached. Fracture short, brittle and granular, taste bitter and acrid and odour not characteristic.The results of macroscopical evaluation are presented in the Table-1.

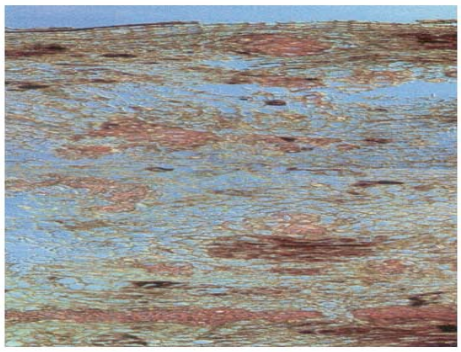
Twig

Bark

Microscopical evaluation

The slides of T.S of bark of plant wasprepared and subjected to microscopical examination. The histology was examined and the observations were recorded. The sectional view of the bark shows that the cork consists of 4-8 layers of tangentially elongated, brick shaped and thin walled parenchymatous cells measuring 28-62 μ in length and 12-44 μ in width. The cork cambium or phellogen is reported to be of single layer; the cells are tangentially elongated, 26-54 μ long 12-38 μ wide and thin walled parenchymatous. Following, the secondary cortex is usually composed of a wide zone of compact, thin walled, medium sized, polygonal to oval parenchymatous cells, which measure 58-138 μ. There are strands of stone cells interspersed in this region. Stone cells are rectangular to somewhat oval with highly thickened and lignified walls bearing numerous simple pits and have wide lumen, measuring 65-145 x 26-58 μ; few stone cells are noticed to possess prismatic crystals of calcium oxalate. Mostly cells of secondary cortex also contain prismatic crystal of calcium oxalate and they are occasionally attached with crystals fibres. Besides, the starch grains are found in few cells of secondary cortex, which are small, simple and oval to round in shape. Laticiferous cells are also studied in the phelloderm containing latex. The medullary rays are bi- or triseriate in the innermost region of secondary phloem and uniseriate medullary rays are rarely observed. The ray cells are thin walled radially elongated parenchyma and individually measures 24-52 x 12-27 μ but gradually increasing in dimension towards outer ends. The secondary phloem is generally represented by sieve tube tissues and companion cells, phloem parenchyma and stone cells. The phloem parenchyma is thin walled, polygonal and is 16-33 μ long and 14-26 μ wide in dimension and few of them have simple starch granules. It is also noticed that some ray cells become sclerenchymatous and attached with stone cells so that it become continuous and extended to 3 to 4 rays in tangential direction. Phloem fibres are found absent (Plate – 1 & 2).

Plate-1
Fig.1: Microphotograph of T.S of Kurchi bark



X 400

Fig.2: A diagrammatic T.S of Kurchi bark

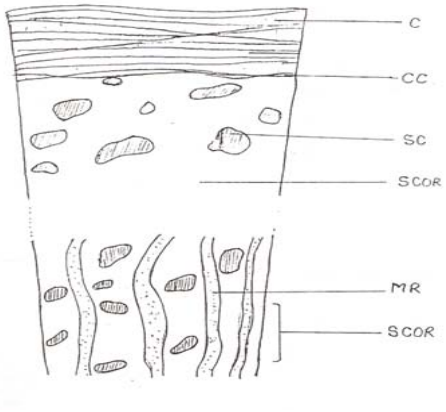
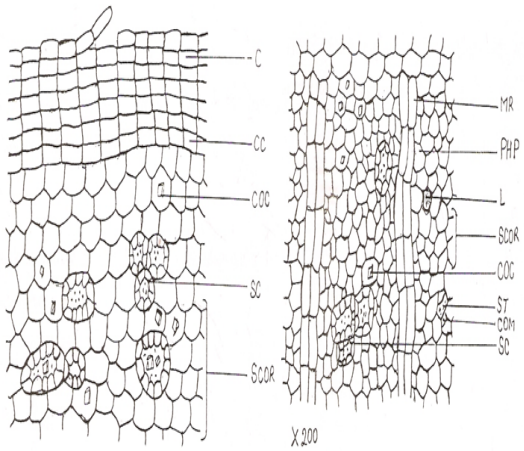


Plate-2: A T.S. section of the bark of Kurchi showing a detailed structure of portion



Abbreviation: T.S. = Transverse section; C=Cork; CC= Cork cambium; COC= Calcium oxalate crystals; COM= Companion cell; L=Laticiferous cells; MR=Medullary rays; PHP= Phloem parenchyma; SC= Stone cell; SCOR= Secondary cortex; ST= Sieve tube

Powder analysis

Powder of the crude drug is brown, coarse and free flowing. The taste is bitter and acrid but odour is not characteristic. Small amount of the powdered material (sieved through 40 mesh) is placed on microscopic slide; mixed with a few drop of 40% w/v aqueous chloral hydrate and heated gently under Bunsen burner. Few drops of 1% alcoholic phloroglucinol are added to this and warmed by mixing one drop of concentrated hydrochloric acid. The slides are then mounted in glycerine and are observed under microscope, which shows the presence of fragments of

cork, secondary cortex having parenchymatous cells, which sometimes associated with stone cells. The groups of stone cells are medium sized, lignified, horizontally striated with broad lumen; ray cells usually found attached with phloem parenchyma; laticiferous cells mostly present free and filled with latex. Cork cambium studied rarely and mostly attached with cork but occasional with phelloderm. Prismatic crystal of calcium oxalate, crystal fibres and starch grains were also studied during the investigation. mostly present free and filled with latex. Cork cambium studied rarely and mostly attached with cork but occasional with phelloderm. Prismatic crystal of calcium oxalate, crystal fibres and starch grains were also studied during the investigation. The reaction of powdered stem bark of *H. antidysenterica* with different chemical reagents was also studied and is depicted in Table-1.

Physicochemical standardization of bark

The air dried, powdered plant materials were subjected for determination of various physicochemical standardization parameter as per the method described in WHO guide lines.

Extractive value

Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with solvent. It is employed for that material for which no chemical and biological assay method exit. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and solvent use. The use of a single solvent can be the means of providing preliminary information on the quality of particular drug. Extractive value also give the information regarding the quality of the drug (whether drug is exhausted or not). The extractive value of Stem Bark of H. Antidysenterica are given in Table-2.

Fluorescence Analysis

The air dried plant materials were subjected to different chemicals and lights. Table-3 showed a detail fluorescence behavior of crude drug powder.

Phytochemical screening

The extracts were subjected to preliminary chemical tests to detect the presence and absence of various phytoconstituents. Alkaloids, carbohydrates, phenolic compounds, flavonoid, proteins and amino acids, saponins and mucilage were absent in petroleum ether, however, resins and lipids were present. Ethanolic extract showed the presence of alkaloids, glycosides, phenolic compounds and tannins. Table-4 showed the presence and absence of various phytoconstitutents in ethanolic extracts. Phytochemical evaluation of the plant extracts may provide the information regarding various types of phytoconstituents present. Presence or absence of particular types of phytoconstituents in the plant of the interest may be helpful, partly in the development of analytical profile and in the differentiation of contravention plants.

Determination of ash values

The percentage of total ash values, water soluble ash, acid insoluble ash were determined. The results noticed were; total ash (6.33%), water soluble ash (2.10%) and acid insoluble ash (3.20%) respectively. and The ash value of any organic material is composed of their non volatile inorganic components. Control incineration of crude drugs result in ash residue consisting of an inorganic material (metallic salt and silica). This value varies within fairly wide limits and is there for an important parameter for the purpose for evaluation of crude drugs. In certain drug, the percentage variation of ash from sample to sample is very small and any marked difference indicates the change in quality. Unwanted parts of drug, some time posses a character that will raise the ash value. Ashing involves an oxidation of the components of the product. A high value is indicative of contamination, substitution, adulterations or carelessness in preparing the crude drug for marketing. The total ash value, acid insoluble ash value, water-soluble ash values were determined as per WHO guide lines. The results and observation are presented in Table-5.

Table-1 Reaction of powdered stem bark of *H. antidysenterica* with different chemical reagents

S.No.	Chemical Reactions	Observation
1.	Conc. sulphuric acid	Reddish Black
2.	Conc. hydrochloric acid	Dark brown
3.	Conc. nitric acid	Yellowish brown
4.	Pot. hydroxide solution (aqueous) (5%)	Dark brown
5.	Sodium hydroxide solution (aqueous) (5%)	Dark brown
6.	Ferric chloride (aqueous)	Greenish blue
7.	Iodine solution	No change
8.	Picric acid	No change
9.	Acetic acid glacial	No change
10.	Powder as such	Brown

Table-2 Extractive Values of *H. antidysenterica* Stem Bark

S.No.	e Solvent	Values in Percentage*
1	Petroleum ether (b.p. 60-80°)	1.60
2	Benzene	1.20
3	Chloroform	0.60
4	Acetone	4.49
5	Ethanol	5.26
6	Distilled water	13.72

* Values are average of three determinations

Table-3 Fluorescence Analysis of Powdered Stem Bark of *H. antidysenterica*

S.No.	Reagents	Colour in Day Light	Observation Under UV Light		
			Modifying Colour	Colour Quality	Radiance Degree
1.	Mounted in Nitrocellulose	Brown	Pinkish brown	Light	Dull
2.	1N NaOH in Methanol	Dark brown	Brown	Dark	Dull
3.	Treated with 1N NaOH in Methanol and mounted in nitrocellulose	Reddish brown	Brown	Light	Dull
4.	1N Hydrochloric acid	Brown	Brown	Light	Bright
5.	Treated with 1N HCl and mounted in nitrocellulose	Dark brown	Pinkish brown	Light	Bright
6.	1N NaOH in water	Dark brown	Brown	Light	Dull
7.	Treated with 1N NaOH in water and mounted in nitrocellulose	Dark brown	Brown	Light	Dull
8.	Diluted Nitric acid (1:1)	Orange yellow	Greenish yellow	Light	Bright
9.	Diluted Sulphuric acid (1:1)	Dark brown	Brown	Light	Dull
10.	Powder as such	Brown	Brown	Light	Dull

Table-4 Preliminary Phytochemical Screening for Detection of Phytoconstituents from Ethanolic Extract of Stem Bark of *H. antidysenterica*

S.No.	Phytoconstituents	Ethanolic Extract
1.	Acidic compounds	-
2.	Alkaloids	+
3.	Carbohydrates	-
4.	Flavonoids	-
5.	Glycosides	+
6.	Phenolic compounds and tannins	+
7.	Proteins and free amino acids	-
8.	Resins	-
9.	Saponins	-
10.	Sterols and triterpenoids	-

+ : Present - : Absent

Table-5 Ash Values of H. antidysenterica Stem Bark

S.No.	Determinants	Values in Percentage
1.	Total ash	6.33
2.	Acid insoluble ash	2.10
3.	Water soluble ash	3.20

Table-6 Distinguishing Microscopic Characters of 'Kurchi' Bark and its Adulterants

Characters	*H. antidysenterica*	*W. tomentosa*	*W. tinctoria*
		Microscopy	
(i)	Stone cells throughout the section, and in phloem region arranged in concentric tangential bands. They are comparatively bigger in size and calcium oxalate present in them.	Stone cells throughout the section and calcium oxalate absent in them.	Bark present only in old plant and stone cells present only in cortical region. Prisms rarely present.
(ii)	Pericyclic fibres present in young but phloem fibres are absent.	Pericyclic fibres are absent but phloem fibres present.	Pericyclic fibres are absent but phloem fibres present.
(iii)	Medullary rays bi to triseriate but very few uniseriate.	Uniseriate and very few bi- or triseriate.	Uniseriate but few biseriate.
(iv)	Starch grains are present.	Starch absent.	Starch absent.
(v)	Cream coloured latex present in laticiferous cells.	Dark brown latex present in laticiferous vessels.	Dark brown granular latex in septate lacitiferous vessels.

Discussion

'Kurchi' is a reputed drug of Indian medicine. The different parts of the plant have been used since antiquity, but the bark of the tree has been more extensively employed as antidysenteric and antidiarrhoeal drug. The bark is also considered to be stomachic, astringent, febrifuge and anthelmintic. The plant is considered useful by tribals (Santhals) in splenic conditions, anaemia, epilepsy, obsteric conditions, spermatorrhoea, haematuria, constipation, stomachic, colic, diarrhoea, and in cholera [11]. 'Kurchi' bark is an ingredient of a number of Ayurvedic formulations, some of which are Amrita Guggulu, Amritarishta, Kirmi Kuthar Rasa, Kirmi Har Quath, Kutajavaleha, Kutajarishta, Kutajashtak Bati etc.

H. antidysenterica has been the most widely accepted source of the drug 'kurchi' throughout the country [12]. But some species of closely related genus *Wrightia*, have also been accepted as the botanical source of its substitute in some parts. The 'kurchi' bark is generally adulterated with bark of *W. tomentosa* and *W. tinctoria*. Kurchi bark, which had early earned its reputation in the European market, lost its value due to adulteration with medicinally inert species of *Wrightia* [1,13,14].

In the pharmacognostical field some of the earlier studies mainly deals with macro and microscopic observation [8,15,16], which are at places contradictory and inadequately informative as the studies falls short on presenting the observation on powder analysis, microchemical test and physical constants. In the present study all these parameters have been supplemented.

Some characters viz. presence of stone cells, crystal fibres and latex are some of the common characters occurring in all the barks. However, there are a number of macro and microscopical differences, which may help in distinguishing them. They are listed in Table-6. The characters of *W. tinctoria* and *W. tomentosa* have been adopted from the earliar work [16,17,19].

The distribution of stone cells, which are the only form of sclerenchymatous tissue in 'kurchi' bark, has been the subject of some controversy. Groups of sclerenchymatous tissue embedded irregularly in the cortex and the phloem region [18]; later it was mentioned this tissue to be arranged in concentric bands [19], the sample studied in former study would have been the species of *Wrightia* as irregular distribution of stone cells is characteristic of *Wrightia* bark.

From the comparative macro and microscopic features presented in Table-6, it is clear that the bark of *H. antidysenterica* can be distinguished from its adulterants (*Wrightia* spp.). Macroscopically, the bark is buff to reddish brown, the taste is acrid and bitter while the odour is not characteristic; fracture is short and granular. Microscopically, the bark can be distinguished by the presence of narrower and tangentially elongated cork cells, concentric tangential bands of stone cells in the phloem containing calcium oxalate prismatic crystals. Phloem fibres are absent, medullary rays are mostly bi or triseriate and some of the ray cells become thick walled and lignified.

Powder analysis of the crude drug revealed the presence of laticiferous cells, cork cells, parenchyma of secondary cortex, prismatic crystal of calcium oxalate, crystal fibres and starch grains. The maceration of 'kurchi' bark was found to certain non-protoplasmic contents like stone cells, calcium oxalate crystals, crystal fibres, starch grains and cells or fragments of cork and secondary cortex. Fluorescence analysis and microchemical colour indicative tests were also carried out with a view to establish the authenticity of the drug. Besides these tests, ash values, extractive values and preliminary phytochemical screening were also carried out. The total ash, acid soluble ash and water insoluble ash were 6.33%, 2.10% and 3.20% respectively. The maximum extractive value was found in distilled water (13.72%) followed by Petroleum ether (60-80°) (1.6%) Benzene (1.20%) Chloroform (0.60%) Acetone (4.49%) and Ethanol (5.26%). Ethanolic extract of the drug was subjected to different tests for detecting the presence of various phytoconstituents present in the drug, which revealed the presence of alkaloids, glycosides, phenolic compounds, and tannins. Tannins have reported the absent in one of the previous study [20] of 'kurchi' bark, while in the present study we found the presence of tannins. Barring the anatomical details and preliminary phytochemical screening, rest of the pharmacognostical parameters have been studied for the first time for the drug 'kurchi'. These details will certainly help in distinguishing the 'kurchi' bark from its adulterants.

The percentage of total alkaloids in *H. antidysenterica* varies with age and girth of plants. The alkaloids contents gets alternatively high and low as the plant gets older and older. The variation in alkaloids content may be due to the presence of peridium, which contain no alkaloids. Further the duration of peridium has been observed as very brief which casts off from time to time and new phellogen tissue develop in the secondary phloem giving rise to the formation of new peridium. Only secondary phloem contains alkaloidal cells [8].

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